

Bacteriological Examination of Ear Swab from University Students

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Abstract: Ear swabs were collected from students complaining of ear infections, revealing a diverse range of bacterial pathogens, including *Pseudomonas* spp, *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp, *Proteus* spp, *Neisseria* spp, *Corynebacterium* spp, *Haemophilus* spp, *Klebsiella* spp, *Saprophytic* spp, *Curtibacterium* spp, *Actinobacter* spp, and *Moraxella* spp. Standard laboratory methods confirmed the presence of these thirteen bacterial species, with *Staphylococcus* spp being the most prevalent. The findings underscore the importance of accurate diagnosis and targeted treatment of ear infections. Moreover, the emergence of these pathogens highlights the need for proper ear hygiene practices and judicious antibiotic use to mitigate the risk of ear infections and curb antibiotic resistance, ultimately informing public health strategies for effective management and prevention.

Keywords: Ear swabs, bacterial pathogens, Standard laboratory methods, judicious antibiotic.

1. INTRODUCTION

Ear infections are a widespread issue affecting both kids and adults, particularly in developing nations. One of the typical signs of an ear infection is ear discharge (Variya et al., 2002).

Roughly 65-330 million people globally deal with ear infections, and around 60% of them experience notable hearing loss (Woodfield et al., 2008).

The human ear's sensitive and complex structure makes it prone to various infections. The ear canal's warm, moist environment is a breeding ground for bacteria. Despite natural defenses, excessive exposure to harmful germs can cause infections like otitis externa and otitis media, leading to symptoms like ear pain, discharge, and hearing issues (Lieberthal et al., 2017)

Ear swabs, also called aural swabs, are important tools used to detect ear infections and find out the bacteria that are causing them. They involve taking a sample from the ear canal for testing in a lab. To make sure the sample is not polluted, special swabs and good aseptic practices are used during the process (Goyal et al., 2017).

The ear, nose, and throat (ENT) are intricately linked and closely associated parts of the body, making it common for health issues in one area to affect the others. As a result, they're typically studied and treated together in a holistic approach. Normally, a wide variety of microorganisms, some of which are harmless under typical conditions, inhabit these areas, playing a role in our overall health and wellbeing (Chibuikwe et al., 2013).

With the global population on the rise, infections are likely to remain a major cause of disease. Upper respiratory infections, in particular, can lead to hearing loss and learning disabilities in kids, affecting their development and future prospects (Obiajurn and Chukuezi 2013).

Research is intensifying on enhancing diagnostic accuracy for ear infections. Studies reveal Gram-negative bacteria, like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, are commonly found. This information is crucial for guiding targeted treatments and improving patient outcomes (Kumar et al., 2018).

The aim of this study was to carry out a bacteriological examination of ear swabs from a group of students

2. MATERIALS AND METHODS

Sample Collection

Ear swabs were taken from 16 microbiology students (9 males, 7 females). The outer ear canal was swabbed, and each sample was placed in a labeled, sterile container with details like name, date, and time. The ear swab was collected from Students of Enugu State University of Science and Technology.

Culture Media Preparation

Media prep was straightforward: blood agar was made by adding 10ml blood to nutrient agar, autoclaved at 121°C for 15mins. High-quality chemicals used throughout.

Sample Analysis

Specimens were incubated on media at 37°C for 48hrs, then colonies were examined, and subcultured for pure isolates.

Gram Stain Procedure

A drop of normal saline was placed on a clean, grease-free slide, and a smear of the culture was made using a sterile wire loop, then heat-fixed. The fixed smear was stained with crystal violet for 60 seconds, washed with distilled water, flooded with lugols iodine for 60 seconds, and washed again. The slide was decolorized with 95% ethanol for 5 seconds, rinsed, stained with safranin for 30 seconds, and rinsed off. Finally, the slide was air-dried and examined under a microscope using a 100x objective lens with a drop of immersion oil (Cheesbrough 2010).

The Biochemical test

This was done according to (Chestbrough 2010).

Citrate Utilization Test

This test identifies bacteria that can use citrate as their only carbon source, indicated by the medium turning purple after incubation at 37°C for 24 hours on Simmons citrate agar.

Catalase Test

This test checks if bacteria produce the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen, indicated by bubbling when hydrogen peroxide is added to the organism on a slide.

Hemolysis Test

Colonies were added to blood agar and incubated at 37°C for 24 hours. Results showed beta hemolysis (complete lysis), alpha hemolysis (partial lysis), or gamma hemolysis (no lysis, considered negative).

Oxidase Test

The oxidase test checks for cytochrome c oxidase. A swab with the sample is pressed onto filter paper with oxidase reagent. A positive result is a dark purple/blue color within 10-30 seconds.

Urease Test

The urease test detects bacteria that produce ammonia from urea. The sample is spread on urea agar and incubated at 35-37°C for 24-48 hours. A positive result is a pink/red color (phenol red indicator).

Methyl Red Test

The Methyl Red Test checks for acid production. The sample is incubated in MR-VP broth at 35-37°C for 48 hours, then methyl red is added. A positive result is a red color (pH below 4.4).

Voges-Proskauer Test

The Voges-Proskauer Test detects acetoin production. The sample is incubated in MR-VP broth, then alpha-naphthol and KOH are added. A positive result is a red color within 10-30 minutes.

The Carbohydrate Fermentation Test

This performs bacteria's ability to ferment sugars (glucose, fructose, lactose, maltose, sucrose, d-mannitol) and produce acid and/or gas. A basal medium with peptone water and sugars was prepared, bacterial isolates were added, and incubated at 37°C for 48 hours. Acid production was shown by a color change with methyl red, and gas production was indicated by a bubble in the Durham's tube.

The Motility Test

This checks if bacteria can move. A loopful of the test organism was placed on a cover slip, inverted, and observed under a microscope (100x) using a hanging drop slide. Movement (excluding Brownian motion) indicated motility; results were classified as motile or non-motile

3. RESULTS

Table 1: Morphological identification of bacterial isolates from ear samples.

Sample	Form	colour	Opacity	margin	elevation	Gram	Shape
Ea	circular	creamy	Opaque	entire	Raised	+ve	cocci
Eb	circular	creamy	Opaque	entire	Raised	+ve	cocci
Ec	circular	creamy	Opaque	entire	Raised	+ve	cocci
Ed	circular	creamy	Opaque	entire	Raised	-ve	diplococci
Ee	circular	creamy	Opaque	Entire	raised	+ve	Rod
Ef	circular	creamy	Opaque	entire	raised	+ve	cocci
Eg	circular	creamy	Opaque	entire	raised	-ve	Rod
Eh	circular	creamy	Opaque	entire	raised	-ve	coccobacilli
Ei	circular	creamy.	Opaque	entire	raised	-ve	Rod
Ej	circular	creamy.	Opaque	entire.	raised.	+ve	cocci
Ek.	circular.	creamy.	Opaque	entire.	raised.	+ve.	cocci
El.	circular.	creamy.	Opaque	entire.	raised	+ve.	cocci
Em.	circular.	creamy.	Opaque	entire.	raised.	-ve.	Rod
En.	circular.	creamy.	Opaque	enti.	raised.	+ve	Rod
Eo	circular	creamy	Opaque	entire	raised	-ve	coccobacilli
Ep	circular	creamy	Opaque	entire	raised	-ve	diplococci

N.B: The above result was for samples cultured on Nutrient agar. Eosin methylene blue agar was used, it showed no growth indicating the absence of coliforms in the samples collected.

Table 2: Biochemical identification of bacteria isolates from ear swab

Sample	Cit	cat	Hae	sp	mot	Ur	Glu	fru	lac	suc	D-man	ox	mr	Probable organism
Ea	-	+	Beta	-	-	+	Ag	A	A	A	A	-	+	<i>Staphylococcus spp</i>
Eb	-	-	Beta	-	-	-	A	A	A	A	A	-	+	<i>Enterococcus spp</i>
Ec	-	-	Beta	-	-	-	A	A	-	-	-	-	+	<i>Streptococcus spp</i>
Ed	-	+	gamma	-	-	-	A	A	-	-	-	+	-	<i>Neisseria spp</i>

Ee	-	+	alpha	-	-	-	A	A	-	A	-	-	+	<i>Corynebacterium spp</i>
Ef	-	-	alpha	-	-	-	A	A	A	A	A	-	+	<i>Streptococcus spp</i>
Eg	+	+	Beta	-	+	-	A	A	-	-	A	+	-	<i>Pseudomonas spp</i>
Eh	-	+	alpha	-	-	-	A	A	-	-	A	+	-	<i>Heamophilus spp</i>
Ei	+	+	gamma	-	-	+	Ag	A	A	A	A	-	-	<i>Klebsiella spp</i>
Ej	-	+	gamma	-	-	-	A	A	-	A	-	-	-	<i>Satapplococcus spp</i>
Ek	-	+	gamma	-	-	-	-	-	-	-	-	-	-	<i>Saprophyticus spp</i>
El	+	-	Beta	-	-	-	A	Ag	-	Ag	Ag	-	-	<i>Streptococcus spp</i>
Em	+	+	gamma	-	+	+	Ag	A	-	A	-	-	+	<i>Proteus spp</i>
En	-	+	gamma	-	-	-	A	A	-	A	-	-	+	<i>Cutibacterium spp</i>
Eo	+	+	Gamma	-	-	-	A	A	-	A	-	-	-	<i>Actinobacters spp</i>
Ep	-	+	gamma	-	-	-	-	-	A	-	-	+	-	<i>Moraxella spp</i>

N.B:cit=citrate,,cat=catalase,hae=haemolysis,sp=spore,mot=motility, ur=urease,glu=glucose,fru=fructose,lac=lactose,suc=sucrose,d-man=d-mannitol,ox=oxidase,mr=methyl red,A=air,Ag=air and gas,

+ =presence of bacteria- =absence of bacteria

Table 3: Distribution of the bacterial isolates from the ear swabs\

Ear swab samples	<i>Pseudomonas spp</i>	<i>Neisseria spp</i>	<i>Streptococcus spp</i>	<i>Corynebacterium spp</i>	<i>Staphylococcus spp</i>	<i>Hemophilus spp</i>	<i>Klebsiella spp</i>	<i>Saprophyticus spp</i>	<i>Cutibacterium spp</i>	<i>Enterococcus spp</i>	<i>Actinobacter spp</i>	<i>Proteus spp</i>	<i>Morexella spp</i>
Ea	-	-	-	-	+	-	-	-	-	-	-	-	-
Eb	-	-	-	-	-	-	-	-	-	+	-	-	-
Ec	-	-	+	-	-	-	-	-	-	-	-	-	-
Ed	-	+	-	-	-	-	-	-	-	-	-	-	-
Ee	-	-	-	+	-	-	-	-	-	-	-	-	-
Ef	-	-	+	-	-	-	-	-	-	-	-	-	-
Eg	+	-	-	-	+	-	+	-	-	-	-	+	-
Eh	-	-	-	-	-	+	-	-	-	-	-	-	-
Ei	-	-	-	-	-	-	-	+	-	-	-	-	-
Ej	-	-	-	-	+	-	-	-	-	-	-	-	-
Ek	-	-	-	-	+	-	-	-	-	-	-	-	-
El	-	-	-	-	-	-	-	-	-	-	-	-	-
Em	-	-	-	-	-	-	-	-	-	-	-	+	-
En	-	-	-	-	-	-	-	-	+	-	-	-	-
Eo	-	-	-	-	-	-	-	-	-	-	+	-	-
Ep	-	-	-	-	-	-	-	-	-	-	-	-	+

4. DISCUSSION

This study focused on examining ear swabs. The isolated organisms were *Pseudomonas* spp, *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp, *Proteus* spp, *Neisseria* spp, *Corynebacterium* spp, *Haemophilus* spp, *Klebsiella* spp, *Saprophytic* spp, *Curtibacterium* spp, *Actinobacter* spp, and *Moraxella* spp. *Staphylococcus* spp had the highest distribution count (3).

This is similar to Ahmed et al (2016), who isolated 52 organisms from ear patients, including *Pseudomonas*, *Streptococcus*, and *Staphylococcus*. This research matches Gideon et al (2017): *Staphylococcal aureus* (31.3%) and *Proteus* species (25.0%) in acute ear discharge. In chronic cases, *Proteus* species dominated (39.1%), followed by *Staphylococcal aureus* (28.3%) .

This study's findings are consistent with Ugwu and Ozochi (2026), who analyzed 150 ear swab samples and found that 43 (28.17%) showed positive bacterial growth, while 107 (71.33%) showed no growth. The positive cultures were distributed among 18 (25.71%) female and 25 (31.25%) male participants. Five bacterial species were identified, including *Escherichia coli* (23.26%), *Pseudomonas* spp (11.63%), *Klebsiella* spp (16.28%), *Streptococcus* spp (18.61%), and *Staphylococcus aureus* (30.23%). Notably, these isolates exhibited a high degree of multidrug resistance to the tested antibiotics. The presence of these bacteria in apparently healthy individuals and their resistance pattern poses a significant public health risk, emphasizing the importance of proper ear hygiene and caution against inserting foreign objects into the ear.

This study's findings are in agreement with Olisaemeka (2012), who conducted bacteriological analysis on 50 ear swabs from consenting male undergraduate students using routine procedures, including pour plating. Thirty-one (31) *Staphylococcus aureus* isolates were identified and further tested for biofilm production and antibiotic susceptibility using Congo red agar and disc diffusion methods.

This study's findings are consistent with Günbey et al (2023), who examined 171 external ear canal culture results from 144 patients at their first admission and 19 patients with recurrent discharge. Pathogenic microorganisms grew in 90 specimens, normal microbial flora in 58, and no growth in 23. *Staphylococcus aureus* was the most common pathogen, followed by *Pseudomonas aeruginosa*. Among Gram-negative bacteria, ampicillin resistance was highest (77.7%)

5. CONCLUSION

The study's bacteriological analysis of ear swabs revealed a diverse range of pathogens, including *Pseudomonas* spp, *Staphylococcus* spp, and *Streptococcus* spp, which significantly contribute to otitis externa and otitis media. These bacterial species are often associated with symptoms such as ear pain, discharge, and hearing loss. The prevalence of these pathogens highlights the need for proper identification and treatment of ear infections. There is a growing concern about antibiotic resistance, emphasizing the importance of judicious antibiotic use. Proper ear hygiene practices, such as avoiding inserting objects into the ear canal and keeping the ears dry, can help mitigate the risk of ear infections and prevent further resistance development. Healthcare professionals should consider these factors when diagnosing and managing patients with ear infections, as timely and effective treatment can significantly improve patient outcomes and reduce the risk of complications. Additionally, public health initiatives aimed at promoting ear health and hygiene can play a crucial role in reducing the burden of ear infections and preventing the spread of antibiotic-resistant bacteria. By adopting a comprehensive approach to ear infection management, we can work towards reducing the impact of these infections on individuals and communities. Effective management of ear infections requires a multifaceted approach that incorporates both preventive and therapeutic strategies. Patients should be educated on the importance of completing antibiotic courses as prescribed and practicing good ear hygiene. Healthcare providers should stay updated on local antimicrobial resistance patterns to inform treatment decisions. Furthermore, research into the epidemiology of ear infections can help identify risk factors and inform public health policies aimed at reducing the incidence of these infections. The role of vaccination in preventing ear infections is also an area of ongoing research, with some vaccines showing promise in reducing the incidence of otitis media. Moreover, public awareness campaigns can help educate individuals on the risks and consequences of untreated ear infections, promoting early seeking of medical attention. By combining these strategies, we can reduce the burden of ear infections and improve health outcomes for individuals and communities.

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